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Molecular mapping of adult plant resistance to *Parastagonospora nodorum* leaf blotch in bread wheat lines ‘Shanghai-3/Catbird’ and ‘Naxos’

Qiongxian Lu ¹, Morten Lillemo *

Department of Plant Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway

¹current address: Science and Technology, Department of Molecular Biology and Genetics, Aarhus University, Forsøgsvej 1, DK-4200 Slagelse, Denmark

*corresponding author, e-mail: morten.lillemo@nmbu.no

Author statement

QL: Conducted the experiments, analysed the data and wrote the manuscript.

ML: Received the research funding, supervised the work and edited the manuscript.

Key message

The field resistance to *Parastagonospora nodorum* leaf blotch in SHA3/CBRD is based on many genes with minor effects.

Abstract

Parastagonospora nodorum leaf blotch is a severe wheat disease in Norway and other regions with humid and rainy climate. It causes grain shriveling and reduced yield in years of epidemics. Shanghai-3/Catbird (SHA3/CBRD), a CIMMYT breeding line, was observed resistant to *P. nodorum* leaf blotch in the field. The objective of the current study was to map the genetic factors related to its resistance. A recombinant inbred line population from a cross between SHA3/CBRD and the susceptible German spring cv. Naxos was tested in field trials over four years (2010, 2011, 2012 and 2013) with natural infection supplied with mist irrigation. Leaf blotch severity was scored together with plant height, heading date and maturity date in these trials. A testing data set was also available from other field trials with the same population. Totally, two major and six minor QTL were detected for leaf blotch resistance. The major QTL on chromosome 3BL with resistance contributed by Naxos was consistent across all environments and explained up to 12% of the phenotypic variation. Another major QTL on 3B with resistance from SHA3/CBRD was significant in 2010, 2013 and the testing data set and explained up to 12% of the phenotypic variation. Minor QTL were detected on 1B, 3AS, 5BS, 5BL, 7A and 7B. The 5BS QTL was likely caused by *Snn3-B1*, with sensitivity contributed by Naxos. The 5BL QTL mapped to the *Tsn1* region, but was likely caused by other mechanisms since both parents were insensitive to ToxA.

Introduction

Parastagonospora nodorum blotch (SNB), caused by *Parastagonospora* (syn. ana, *Stagonospora*; teleo, *Phaeosphaeria*) *nodorum* (Berk.) Quaedvlieg, Verkley & Crous (Quaedvlieg et al. 2013), is a necrotrophic fungal disease affecting leaves and glumes. It occurs in many wheat production regions around the world with a temperate and rainy climate, and causes severe grain shriveling and substantial yield losses under epidemics (Solomon et al. 2006). Yield reductions have been reported up to 31% and even around 40% (Bhathal et al. 2003; Eyal et al. 1987).

P. nodorum used to be the dominant leaf blotch pathogen of wheat in the UK during the 1970s and 1980s (Bearechell et al. 2005) and is still found to be the main causal agent for leaf blotch in Norway (Ficke 2010) though *Septoria tritici* blotch (caused by *Zymoseptoria tritici*) became dominant in the UK recently (Bearechell et al. 2005) and has increased in importance in Northern Europe (Scharen 1999). Under natural infection in the field, symptoms of SNB can be very difficult to distinguish from other coexisting leaf blotch diseases such as tan spot (caused by *Pyrenophora tritici-repentis*) and *S. tritici* blotch. Consequently, severity of the leaf blotch disease complex is usually recorded.

To prevent yield losses, intensive fungicide application is inevitable which in fact poses a high selection pressure for fungicide resistance (Fraaije et al. 2005) and added production costs to the farmers. Breeding resistant wheat varieties in conjunction with effective cultural practices is considered to be the most cost-effective and environmentally benign way to manage leaf blotch.

Due to limited understanding of the resistance genetics and specific host-pathogen interactions, breeding for resistance to SNB has made slow progress. In the 1990s, crude extract of *S. nodorum* was found to induce the disease *in vitro* and subsequently applied to screen the resistance level of breeding material in early generations (Keller et al. 1994; Wicki et al. 1999). More recently, studies have shown that *S. nodorum* and other necrotrophic leaf blotch pathogens interact with their hosts in an inverse gene-for-gene manner based on necrotrophic effectors (NEs, also known as host-selective toxins). So far, at least six NEs (SnToxA, SnTox1, SnTox2, SnTox3, SnTox4 and SnTox5) and

corresponding host sensitivity loci (*Tsn1*, *Snn1*, *Snn2*, *Snn3*, *Snn4* and *Snn5*) have been described for the wheat - *P. nodorum* pathosystem (Francki 2013; Friesen et al. 2012; Friesen and Faris 2010). This has opened up new possibilities in resistance breeding by identification and elimination of host sensitivity loci.

Identification of molecular markers closely linked to sensitivity/resistance loci is essential in marker assisted selection for SNB resistance. Many QTL have been identified on the chromosomes such as 1B, 2B, 2D, 4B, 5A, 5B, 5D, 6A and 7A for seedling resistance (Adhikari et al. 2011; Arseniuk et al. 2004; Czembor et al. 2003; Faris and Friesen 2009; Friesen et al. 2012; Friesen et al. 2009; Liu et al. 2004b; Reszka et al. 2007) and 1B, 2A, 2D, 5A, 5B and 7A for adult plant leaf resistance (Aguilar et al. 2005; Friesen et al. 2012; Friesen et al. 2009; Shankar et al. 2008).

Evidence has been provided that QTL on 1AS, 1BS, 2DS, 4BL, 5BS, and 5BL are corresponding to the sensitivity loci *Snn4*, *Snn1*, *Snn2*, *Snn5*, *Snn3* and *Tsn1*, respectively (Abeysekara et al. 2010; Friesen et al. 2012; Friesen et al. 2007; Friesen et al. 2008; Liu et al. 2004a; Liu et al. 2006; Liu et al. 2004b). These QTL were detected after inoculation with single isolates at the seedling stage and accordingly accounted for large proportions of the variation. QTL from other studies, however, have not been reported to be associated with any known sensitivity loci. Most of them explained less than 20% of the phenotypic variation (reviewed by Francki 2013).

A recombinant inbred line (RIL) population from a cross between SHA3/CBRD (resistant) and Naxos (susceptible) was initially developed for genetic analysis of Fusarium head blight and powdery mildew resistance (Lu et al. 2012; Lu et al. 2013). In some of the powdery mildew trials that were exposed to rainy conditions during grain filling, segregation for leaf blotch resistance was observed.

To investigate the genetic basis of the SNB resistance segregating in the RIL population, field testing was conducted in mist-irrigated hillplot nurseries over four years. The objectives were to: 1) identify the main genetic factors associated with SNB resistance in the RIL population, 2) determine whether any of these factors correspond to known NE-

sensitivity loci, and 3) compare with results of other QTL studies of seedling and adult plant resistance to SNB.

Material and methods

Plant materials

A RIL population of 181 F₆ lines was developed by single seed descent from the cross SHA3/CBRD x Naxos, which was initially developed for genetics analysis of Fusarium head blight and powdery mildew resistance (Lu et al. 2012; Lu et al. 2013). SHA3/CBRD is a spring type breeding line from CIMMYT with the pedigree ‘Shanghai-3//Chuanmai 18/Bagula’ and selection history “-0SHG-6GH-0FGR-0FGR-0Y”. Naxos, a German spring variety, was developed by Strube GmbH & Co.KG from the cross ‘Tordo/St.Mir808-Bastion//Minaret’. In naturally infected field trials in Norway, SHA3/CBRD showed high resistance to SNB, whereas Naxos was susceptible. Toxin assays showed that both parents are insensitive to SnToxA and SnTox1, whereas Naxos is sensitive and SHA3/CBRD insensitive to SnTox3 (Tim Friesen, pers. comm.).

Field trials

Field testing was conducted with a subset of 168 RILs, which excluded a few lines with very late maturity or poor seed set. The RILs were tested together with their parents in hillplot trials naturally infected with *P. nodorum* during the 2010, 2011, 2012 and 2013 seasons at Vollebekk Research Station in Ås, Norway. The symptoms of SNB are difficult to distinguish from other foliar diseases such as tan spot and *S. tritici* blotch, so actually the leaf blotch complex was scored in this study. However, PCR assays of randomly collected leaf samples from the 2010 and 2011 field trials, and microscopic inspection of leaf samples from 2012 and 2013 confirmed that *P. nodorum* was the dominating pathogen (data not shown). Field trials were carried out in an alpha lattice design with two replications. The trials were mist irrigated for 5 min every half hour at

daytime to promote leaf blotch epidemics and avoid competing diseases like powdery mildew.

Leaf blotch severity was assessed visually as the percentage of diseased leaf area based on the whole canopy. Two scores were registered in 2010, 2011 and 2013, one in 2012 based on the disease development on susceptible checks. Typically, the first score was done when the most susceptible lines had reached 70-80% severity (about three weeks after heading), and the second score about one week later when some lines had already reached 100% severity. Developmental traits reported to affect leaf blotch rating (Aguilar et al. 2005; Tommasini et al. 2007) were also recorded. Maturity date was scored in all four years, while heading date and plant height were recorded in 2011, 2012 and 2013.

Additionally, leaf blotch data obtained from three powdery mildew experiments (Lu et al. 2012) at Vollebekk in 2009 and Staur research farm (close to Hamar, Norway) in 2009 and 2010 were used as testing data. In those field seasons, the plants were subjected to rainy conditions during the grain-filling stage which stopped powdery mildew development and promoted leaf blotch. Leaf blotch severity was scored once in all three experiments in the same manner as described above. To avoid possible confounding effects from powdery mildew, these data were only used to test the detected QTL from the mist-irrigated leaf blotch trials.

Statistical analysis

Analyses of variance were performed using the PROC GLM procedure in SAS v. 9.2 (SAS Institute Inc.). Heritability (broad sense) was estimated from the ANOVA information using the formula $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_E^2 / r)$ within a year and the formula $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{g \times y}^2 / y + \sigma_E^2 / ry)$ across years, where σ_g^2 is genetic variance, $\sigma_{g \times y}^2$ is genotype-by-year interaction, σ_E^2 is error variance, y is number of years, and r is number of replicates. The mean leaf blotch severity of each line was estimated in SAS with the

LSmeans statement in PROC MIXED. The Pearson correlation coefficients were calculated using the PROC CORR procedure in SAS.

Genetic map construction

Initial QTL mapping was carried out based on the existing linkage map for Fusarium head blight and powdery mildew studies (Lu et al. 2012; Lu et al. 2013). This linkage map was constructed from the genotypic data of 181 lines including 283 DArT and 271 SSR loci. Later, 13 markers (16 loci) close to *Tsn1*, *Snn1*, *Snn2*, *Snn3-B1*, *Snn3-D1* and *Snn4* (Abeysekara et al. 2009; Friesen and Faris 2010; Reddy et al. 2008; Zhang et al. 2009; Zhang et al. 2011) were added. Excluding three redundant loci, the final genetic map was developed with the software JoinMap v. 3.0 (van Ooijen and Voorrips 2001). Map distances were based on the Kosambi function with minimum LOD score of 2. Consensus map information was used to assign linkage groups to chromosomes.

QTL analysis

QTL mapping was performed mainly by MapQTL v6.0 (Van Ooijen 2009). In order to determine the covariates in MapQTL, leaf blotch severity in each environment was regressed against the means of days to heading (DHm), days to maturity (DMm) and plant height (PHm), all of which were significant. In the QTL analyses, the corresponding DH, DM and PH from the same leaf blotch experiment were used as covariates. If the corresponding data was not scored, the overall means were used instead. Interval mapping (IM) was first run with DH, DM and PH as covariates with the LOD threshold 3.0. All the significant QTL were used as initial cofactor set to determine the cofactors for multiple QTL mapping (MQM) with the backward elimination procedure in MapQTL ($\alpha = 0.02$). Both MQM and restricted MQM (rMQM) mapping were conducted with cofactors and covariates. However, QTL results from MQM and rMQM were no better than those from IM based on the LOD curve; some QTL became even less significant. A possible reason for this could be that cofactors were not close to the QTL peak due to limited map resolution, which could affect the power of QTL detection.

Two other QTL mapping softwares, PlabQTL v1.2 (Utz and Melchinger 2003) and QTL IciMapping v3.2 (Li et al. 2008), were also tried in order to challenge the results from MapQTL. To make the results comparable across the softwares, adjusted leaf blotch severities were used in PlabQTL and IciMapping. These were calculated based on the covariate estimation in MapQTL by subtracting the estimated effects of the associated traits from observed severity (Table S1).

Multiple regressions with significant QTL were run in PlabQTL using the adjusted leaf blotch severities. By eliminating the non-significant QTL at each round, multiple regressions were re-run until all the QTL in the model were significant. Genetic map drawing and QTL marking were conducted by the software MapChart v.2.1 (Voorrips 2002).

Single marker analyses were conducted between markers for known sensitivity loci and adjusted leaf blotch severities with Pearson's correlation method.

Results

Phenotypic evaluation

A broad variation was observed for both leaf blotch severity (Fig.1) and associated traits (Fig.S1) in the RIL population. The disease severity ranged from 0 to 100% in all four years, favored by mist irrigation. However, the severity in 2012 had higher standard deviation but similar severity range.

Highly significant correlation coefficients were observed for leaf blotch severities across 2010, 2011 and 2013, while lower between 2012 and other years (Table 1). Despite significant genotype by environment interaction and moderate correlation coefficients ($r = 0.47-0.78$), a considerable heritability of 0.84 was still observed across years (Table 2).

Leaf blotch severity showed significant negative correlations with DH ($r = -0.39$ to -0.61) and DM ($r = -0.43$ to -0.62) at similar magnitudes, and with PH ($r = -0.25$ to -0.43) at a less magnitude.

Leaf blotch severities obtained from powdery mildew trials were used as a testing data set. Severities were consistent across years ($r = 0.64$ to 0.75) and even showed high considerable correlation coefficients ($r = 0.30$ to 0.74) with data from the mist irrigated leaf blotch trials.

QTL mapping results

Seven significant QTL and one putative QTL were detected in the four leaf blotch experiments at Vollebekk (Table 3). Six out of these eight QTL were significant in the testing data.

The most consistent QTL was detected on 3BL near the marker *wPt-4933*, explaining up to 12% of the phenotypic variation. It was significant in all environments, and the resistance was contributed by Naxos. In the testing data, this QTL was significant in two environments and with the mean data. Another QTL, with resistance from SHA3/CBRD, was found on 3B explaining over 11% of the phenotypic variation. This QTL was significant in two environments and verified with the testing data of Vollebekk in 2009 and the mean.

The QTL on 1B, 5BL and 7A were detected in two environments and the mean data, explaining from 2 to 8% of the total phenotypic variation. The 5BL QTL is near the *Tsn1* marker *fcp1*. The LOD curve of the 5BL QTL peaked at two neighboring positions in two years with the highest peak at 30 cM in 2010 and 42 cM in 2011. They are considered the same QTL due to overlapping confidence intervals. The rest of the QTL were detected in either one or two environments and explained less than 10% of the variation.

Effects of NE sensitivity loci

Of the known NE sensitivity loci, data were obtained for a total of 10 markers linked to *Tsn1*, *Snn1*, *Snn2*, *Snn3-D1*, *Snn4* and *Snn5*. Other markers were either monomorphic or did not map to the expected chromosomes and were not considered for this analysis. This

included all six tested markers for *Snn3-B1* (BE606637, BE446811, gwm234, cfd20, wmc149 and wmc728), and the most closely linked reported marker for *Snn5* (wmc349, Friesen et al. 2012).

The *Tsn1* marker *fcp1* was associated with adjusted leaf blotch severity. The effect was significant in 2010 and for the mean data across years. Another marker, *barc163* reported to be linked to *Snn5* showed significant correlation only with leaf blotch severity in 2010. In contrast, markers linked to other NE loci had no effect in any of the environments (Table 4).

Discussion

Phenotypic data

This study is based on natural infection. The *P. nodorum* pathogen population can be expected to differ from year to year, which would lead to different interactions between sensitivity loci and pathogen races. The different reaction patterns can accordingly result in the detection of different genetic factors in different years. The pathogens for *S. tritici* blotch and tan spot could also have added complexity to the study, although *P. nodorum* was by far the most dominating leaf blotch pathogen in our four trials.

Highest standard deviation was observed in 2012 but with similar severity range, indicating that fewer genes were responsible for the disease in that year. This was also supported by the QTL results in which only one QTL was detected above the significance threshold.

The high negative correlations between severity and developmental traits complicates the QTL analysis based on original disease scores, and makes it difficult to distinguish true resistance QTL from those caused by the confounding effects of earliness and plant height. There are two possible solutions. Firstly, to run QTL analysis on leaf blotch severity and associated traits separately; QTL for disease resistance without coincident QTL for associated traits are more likely real (Lu et al. 2013). Alternatively, the QTL analysis can be based on adjusted leaf blotch severities, which are calculated by

subtracting the fitted leaf blotch value from the observed leaf blotch scores. Here we used the latter, which is equivalent to the mapping strategy in MapQTL by running the original data with plant height, days to heading and days to maturity as covariates. In addition, it can help to avoid that some under-the-threshold QTL for associated traits were not recognized as confounding factors to leaf blotch. The adjusted leaf blotch data were not correlated with DH, DM or PH, which indicates that this adjustment was effective in avoiding the confounding effects from associated traits.

QTL Mapping

A total of eight QTL were detected in this study. Although we cannot rule out that some of the signal from the phenotypic data could be caused by other leaf blotch pathogens present at low frequencies in the field, we find it likely that the reported QTL are involved in resistance/susceptibility to *P. nodorum*. Firstly, monitoring of the pathogen population in the field trials either by PCR or microscopic inspection confirmed that *P. nodorum* was the dominating pathogen in all seasons. Secondly, preliminary data from our testing with single isolates of *P. nodorum* at the seedling stage in the same RIL population have identified corresponding LOD peaks at most of the adult plant resistance QTL reported in this study.

Two QTL were detected on 3B. The one on 3BL appears to be novel and showed consistent effect across all the environments and significant effect with the testing data, which indicates its potential as a resistance source. The other 3B QTL was located near the centromere, at a similar position as a seedling resistance QTL reported by Reszka et al (2007) and Adhikari et al (2011). A tan spot resistance QTL has also been reported close to the centromere on 3B (Faris and Friesen 2005).

The 5BL QTL was located close to the *Tsn1* locus which has been cloned, and *Tsn1*-*ToxA* is the best studied interaction in both the wheat - *P. tritici-repentis* and wheat - *P. nodorum* pathosystems (Faris et al. 2010). However based on toxin assay, both parents were insensitive to *ToxA* and did not segregate at this locus (Tim Friesen, pers. comm.). Additionally, the flanking markers *fcp620* and *fcp394* (Zhang et al. 2009) are

monomorphic in the RIL population, and both parents carry the alleles associated with insensitivity (data not shown). It indicates that this 5BL QTL is probably a different, but closely linked locus for NE sensitivity or other resistance mechanism. Other studies also reported QTL on 5BL responsible for leaf blotch. Czembor et al. (2003) found a seedling QTL on 5B near the marker *barc32* explaining 30% of the phenotypic variation. In the present study, the 5BL QTL peaked more distal in 2011 and for the mean data than in 2010. Such difference was also observed in a RIL population in which the position of 5BL QTL were different (Francki et al. 2011). It indicates that there might be a complex of multiple genes on 5BL involved in the susceptibility or resistance mechanism to the pathogen.

The 5BS QTL was likely caused by *Snn3-B1*, although the identity of this QTL could not be confirmed since all the most closely linked published markers to this locus were monomorphic. However, further evidence comes from toxin assays showing that Naxos is sensitive to Tox3 while SHA3/CBRD is insensitive (Tim Friesen, pers. comm.). However, the effect of this QTL was small and only detected in 2010.

The 1B QTL detected here was peaking about 10 cM distal to a QTL responsible for flag leaf resistance in the BR34 x Grandin population on 1BS (Friesen et al. 2009), 20 cM from a QTL responsible for the glume blotch resistance in Forno x Oberkulmer (Aguilar et al. 2005) according to Somers' consensus map (2004). Based on the marker analysis (Table 4), this QTL is likely not caused by *Snn1*. The resistance at this locus might be the result of a sensitivity locus on 1RS in SHA3/CBRD, which carries the 1B/1R translocation (Lu et al. 2012). Or it could possibly be from Naxos through other resistance mechanism.

The QTL close to the centromere on 7B was mapped at a similar position as a leaf blotch QTL with resistance from Forno (Aguilar et al. 2005). A glume blotch QTL was also detected in this 7B centromeric region (Schnurbusch et al. 2003; Shankar et al. 2008). There might be more than one QTL in this region due to the limited resolution near the centromere, which led to overlapping peaks in 2012. The 3AS QTL mapped to the same region as the tan spot resistance gene *tsr4* (Tadesse et al. 2010). Other minor QTL were either novel or could not be compared.

According to the marker analysis of known NE sensitivity loci, the *Snn5*-linked marker *barc163* was significant in 2010. However, the most closely linked marker to *Snn5* – *wmc349* was monomorphic, and no QTL was detected on 4B in the QTL analysis, either in single years or for the mean across years. We can therefore conclude that *Snn5* did not show any important effect in this population.

Generally, QTL caused by NE sensitivity show large effect at the seedling stage when plants are inoculated with single isolates (Abeysekara et al. 2009; Friesen et al. 2009; Liu et al. 2004b). However, at the adult plant stage in the field, when inoculated with the same isolate, these effects became smaller (Friesen et al. 2009). Adding the diverse and dynamic natural pathogen population in our study, such reduced effects will inevitably be diluted by other pathogen isolates with different NEs. This is supported by the QTL mapping results, which showed that many minor QTL were involved rather than a few major ones.

Conclusion and prospects

In this study, genetic analysis showed that resistance in SHA3/CBRD was controlled by one major QTL on 3B and two minor QTL on 5BS and 5BL. The susceptible parent Naxos contributed one major QTL on 3BL and four minor QTL. The minor QTL on 5BS was likely a result of Naxos carrying the sensitivity allele of *Snn3*. Less resistance loci were detected contributed by SHA3/CBRD than Naxos, which indicates that the resistance in SHA3/CBRD is controlled by many genes with minor effects. The markers linked to the QTL on 3B and 3BL could have the potential for application in marker assisted selection.

These QTL were identified under natural infection with a complex pathogen population which had different virulence factors. It is important to clarify the nature of the interaction between these QTL and the pathogen. Hence, more detailed investigation of this population will be our further work. Differential isolates and NE filtrates will be applied to test the population and parents for specific interactions at the seedling stage, and the map resolution around important QTL will be refined by adding more markers.

Ethical standards

All experiments included in this study comply with the current laws of the country in which they were performed.

Conflict of interest

The authors declare no conflict of interest.

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Figure captions

Fig. 1 Frequency distributions of leaf blotch severity in the SHA3/CBRD x Naxos RIL population. Leaf blotch severity is shown along the X-axis and number of lines in each category on the Y-axis. The average severities of SHA3/CBRD (S) and Naxos (N) are indicated by arrows.

Fig. 2 Chromosomes with significant QTL, with corresponding LOD curves obtained from interval mapping (IM). If there was no QTL detected based on the mean, the environment with significant QTL effect was marked instead with the year at the end of the QTL name. Genetic distances are shown in centimorgans to the left of the chromosomes. A threshold of 3.0 is indicated by a dashed vertical line in the LOD graphs. The approximate positions of centromeres are indicated by arrows.

Table 1 Pearson correlation coefficients among leaf blotch severities and developmental trait means in the SHA3/CBRD x Naxos RIL population

| | Leaf blotch severities from mist-irrigated leaf blotch trials | | | | Leaf blotch severities from powdery mildew trials | | | | |
|------------------|---|----------|----------|----------|---|------------------|----------|------------|------------|
| | 2010 | 2011 | 2012 | 2013 | Days to heading | Days to maturity | 2009 Ås | 2009 Hamar | 2010 Hamar |
| 2010 | | | | | | | 0.73*** | 0.69*** | 0.67*** |
| 2011 | 0.73*** | | | | | | 0.62*** | 0.64*** | 0.48*** |
| 2012 | 0.47*** | 0.50*** | | | | | 0.36*** | 0.41*** | 0.30*** |
| 2013 | 0.78*** | 0.75*** | 0.47*** | | | | 0.73*** | 0.74*** | 0.61*** |
| Days to heading | -0.45*** | -0.45*** | -0.39*** | -0.61*** | | | -0.47*** | -0.39*** | -0.41*** |
| Days to maturity | -0.56*** | -0.62*** | -0.43*** | -0.56*** | 0.65*** | | -0.52*** | -0.46*** | -0.44*** |
| Plant height | -0.43*** | -0.36*** | -0.25* | -0.38*** | 0.01 | 0.09 | -0.18 | -0.24* | -0.31*** |
| 2009 Ås | | | | | | | | 0.75*** | 0.64*** |
| 2009 Hamar | | | | | | | | | 0.66*** |

***<0.0001, **<0.001, *<0.01

Table 2 Analysis of variance for leaf blotch severity and associated traits and their heritabilities in the SHA3/CBRD x Naxos RIL population

| Traits | Source | DF | Mean | | P-Value | Heritability |
|------------------|---------------|-----|----------|---------|---------|--------------|
| | | | Square | F Value | | |
| Leaf blotch | Genotype | 167 | 1427.83 | 6.11 | <.0001 | 0.84 |
| | Year | 3 | 17590.13 | 75.32 | <.0001 | |
| | Genotype*Year | 501 | 233.55 | 2.22 | <.0001 | |
| | Rep(Year) | 3 | 1761.60 | 16.76 | <.0001 | |
| | Block(Rep) | 28 | 166.66 | 1.59 | 0.0294 | |
| | Error | 590 | 105.08 | | | |
| Days to heading | Genotype | 167 | 35.52 | 9.11 | <.0001 | 0.89 |
| | Year | 2 | 10508.53 | 2694.70 | <.0001 | |
| | Genotype*Year | 334 | 3.90 | 2.50 | <.0001 | |
| | Rep(Year) | 2 | 0.33 | 0.21 | 0.8087 | |
| | Block(Rep) | 28 | 2.85 | 1.83 | 0.0067 | |
| | Error | 471 | 1.56 | | | |
| Days to maturity | Genotype | 167 | 63.86 | 2.84 | <.0001 | 0.65 |
| | Year | 3 | 16105.58 | 716.85 | <.0001 | |
| | Genotype*Year | 501 | 22.47 | 2.59 | <.0001 | |
| | Rep(Year) | 3 | 80.33 | 9.26 | <.0001 | |
| | Block(Rep) | 28 | 15.86 | 1.83 | 0.0061 | |
| | Error | 638 | 8.67 | | | |
| Plant height | Genotype | 167 | 376.43 | 10.78 | <.0001 | 0.91 |
| | Year | 2 | 7362.10 | 210.90 | <.0001 | |
| | Genotype*Year | 334 | 34.90 | 1.25 | 0.0136 | |
| | Rep(Year) | 2 | 246.01 | 8.80 | 0.0002 | |
| | Block(Rep) | 28 | 29.81 | 1.07 | 0.3762 | |
| | Error | 473 | 27.97 | | | |

Table 3 QTL for leaf blotch severity in the SHA3/CBRD x Naxos RIL population. The percentage of explained phenotypic variation (R^2) in the multiple regression models is shown. QTL are listed if they were over the LOD threshold of 3 in at least in one environment and showed significant contribution in the multiple regression models. QTL detected above the LOD threshold in the corresponding environment are indicated in bold.

| Chr. | Closest marker | Mist-irrigated leaf blotch trials | | | | | R source | Testing dataset | | | |
|-------------|----------------|-----------------------------------|-------------|------------|------------|------|-----------|-----------------|------------|------------|------|
| | | 2010 | 2011 | 2012 | 2013 | Mean | | 2009 Ås | 2009 Hamar | 2010 Hamar | Mean |
| 1B | wmc619 | | 2.3 | | 6.3 | 3.6 | Naxos | | | 7.1 | |
| 3AS | gwm2 | 9.1 | | | 7.6 | | Naxos | 4.4 | | | |
| 3B | wPt-4127 | 11.3 | | | 6.1 | | SHA3/CBRD | 11.7 | | | 2.6 |
| 3BL | wPt-4933 | 8.0 | 11.7 | 2.7 | 7.2 | 9.0 | Naxos | 3.4 | 3.1 | | 2.5 |
| 5BS | wPt-5346 | 3.7 | | | | | SHA3/CBRD | | | | |
| 5BL | fcp1 | 6.1 | 2.6 | | | 5.8 | SHA3/CBRD | 6.5 | | | |
| 7A | wmc603 | | 3.3 | | 8.3 | 4.6 | Naxos | | | | |
| 7B | wPt-0963 | 2.9 | | 8.8 | | | Naxos | | | | 3.4 |
| R^2 total | | 34.4 | 17.4 | 10.9 | 29.0 | 23.1 | | 21.0 | 3.1 | 7.1 | 8.5 |

Table 4 Pearson's correlation coefficients between marker alleles for known toxin sensitivity loci and adjusted leaf blotch severities

| Locus | Chr. | Marker | 2010 | 2011 | 2012 | 2013 | Mean |
|----------------|------|----------|-------|-------|-------|-------|-------|
| <i>Tsn1</i> | 5BL | fcp1 | 0.23* | 0.12 | 0.14 | 0.14 | 0.22* |
| <i>Snn1</i> | 1BS | psp3000a | 0.12 | -0.03 | -0.02 | -0.12 | -0.04 |
| <i>Snn2</i> | 2DS | cf51 | -0.13 | -0.06 | -0.12 | -0.03 | -0.09 |
| | | cf56 | -0.11 | 0.03 | -0.04 | 0.06 | 0.01 |
| <i>Snn3-D1</i> | 5DS | cf18 | 0.1 | 0.06 | 0.06 | -0.05 | 0.02 |
| | | gwm190 | 0.08 | -0.01 | 0.04 | -0.06 | 0.01 |
| <i>Snn4</i> | 1AS | BG262267 | 0.04 | 0.09 | 0.04 | -0.02 | 0.03 |
| <i>Snn5</i> | 4BS | gwm375 | 0.18 | 0.10 | -0.05 | 0.09 | 0.11 |
| | | barc163 | 0.21* | 0.08 | -0.09 | 0.10 | 0.09 |
| | | wmc679 | 0.14 | 0.11 | -0.05 | 0.03 | 0.07 |

* $P < 0.01$

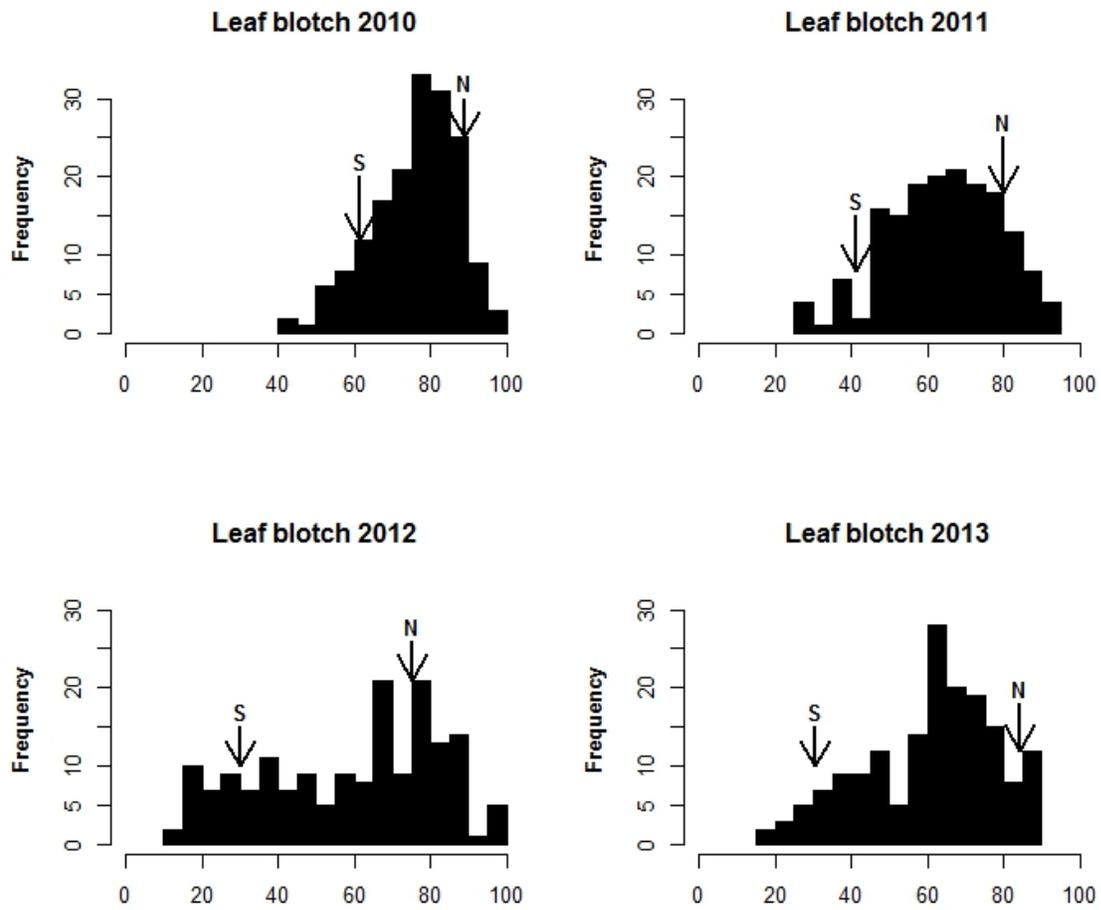
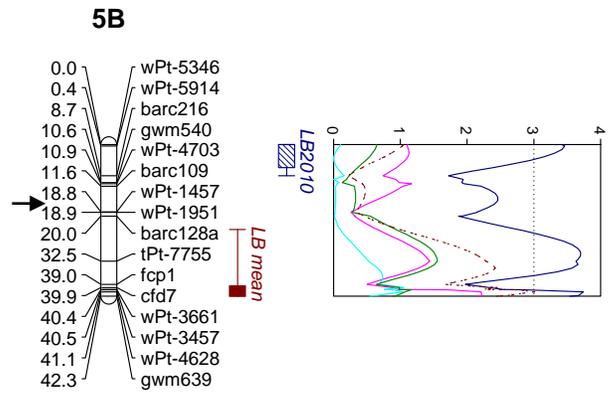
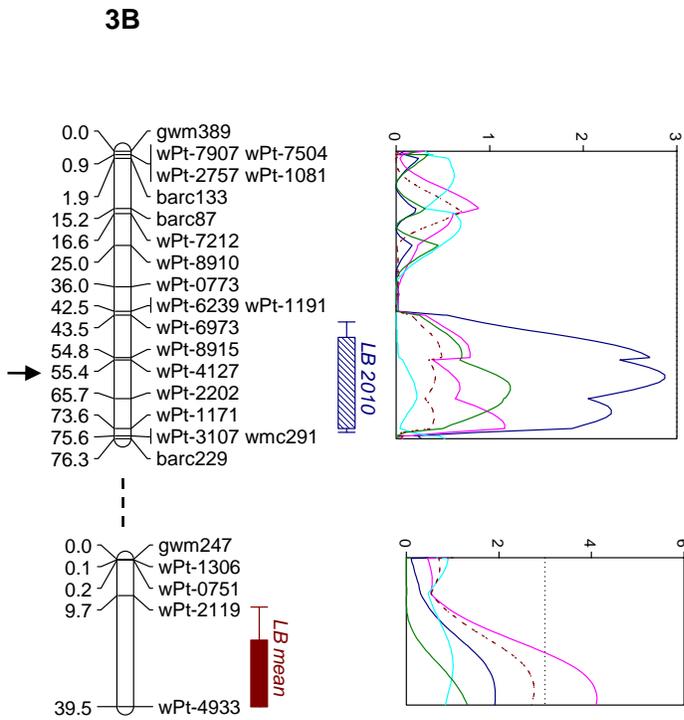
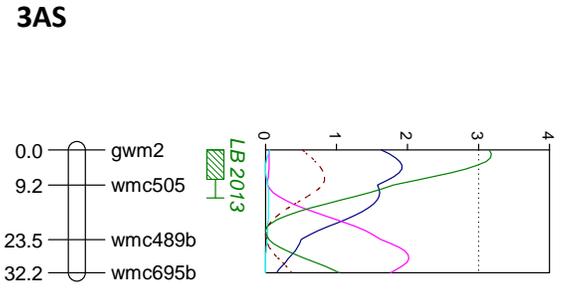
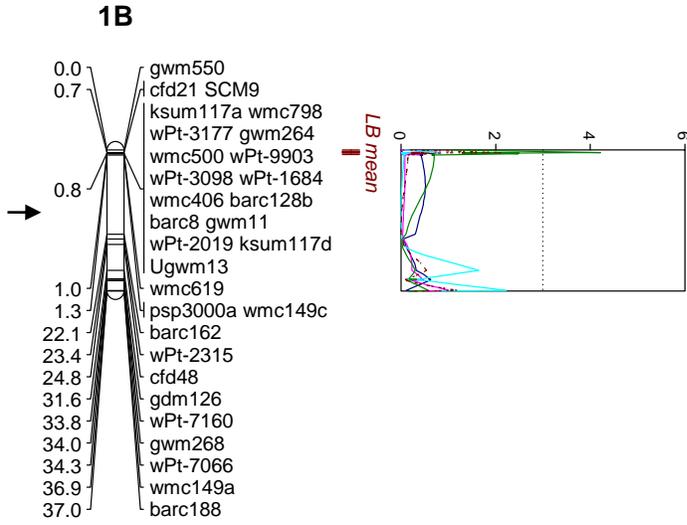


Fig. 1 Frequency distributions of leaf blotch severity in the SHA3/CBRD x Naxos RIL population. Leaf blotch severity is shown along the X-axis and number of lines in each category on the Y-axis. The average severities of SHA3/CBRD (S) and Naxos (N) are indicated by arrows.



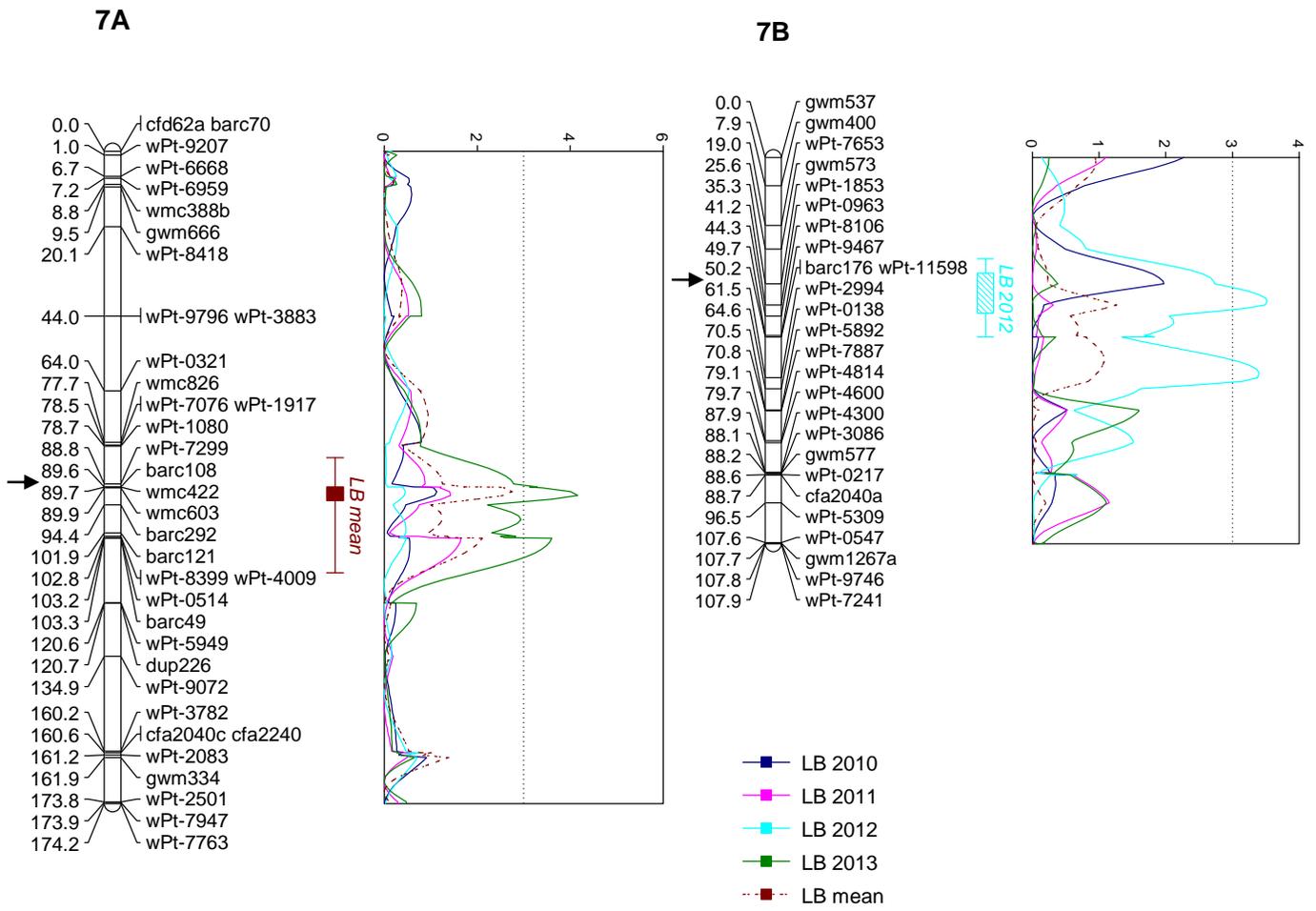


Fig. 2 Chromosomes with significant QTL, with corresponding LOD curves obtained from interval mapping (IM). If there was no QTL detected based on the mean, the environment with significant QTL effect was marked instead with the year at the end of the QTL name. Genetic distances are shown in centimorgans to the left of the chromosomes. A threshold of 3.0 is indicated by a dashed vertical line in the LOD graphs. The approximate positions of centromeres are indicated by arrows.

Table S1 The regression models used to adjust the observed leaf blotch severities for the effects of associated traits. DH = days to heading; DM = days to maturity, PH = plant height.

| Year | Regression model | R ² |
|------|---|----------------|
| 2010 | $y' = 307.1069 - 1.62852DHm - 0.96315DM10 - 0.33288PHm$ | 55.59% |
| 2011 | $y' = 419.1148 - 2.03577DH11 - 1.70988DM11 - 0.64093PH11$ | 44.65% |
| 2012 | $y' = 412.755 - 3.34671DH12 - 0.77202DM12 - 0.79143PH12$ | 23.51% |
| 2013 | $y' = 351.4052 - 1.92754DH13 - 1.37893DM13 - 0.67627PH13$ | 63.59% |
| mean | $y' = 420.725 - 1.68383DHm - 1.97263DMm - 0.62571PHm$ | 56.89% |

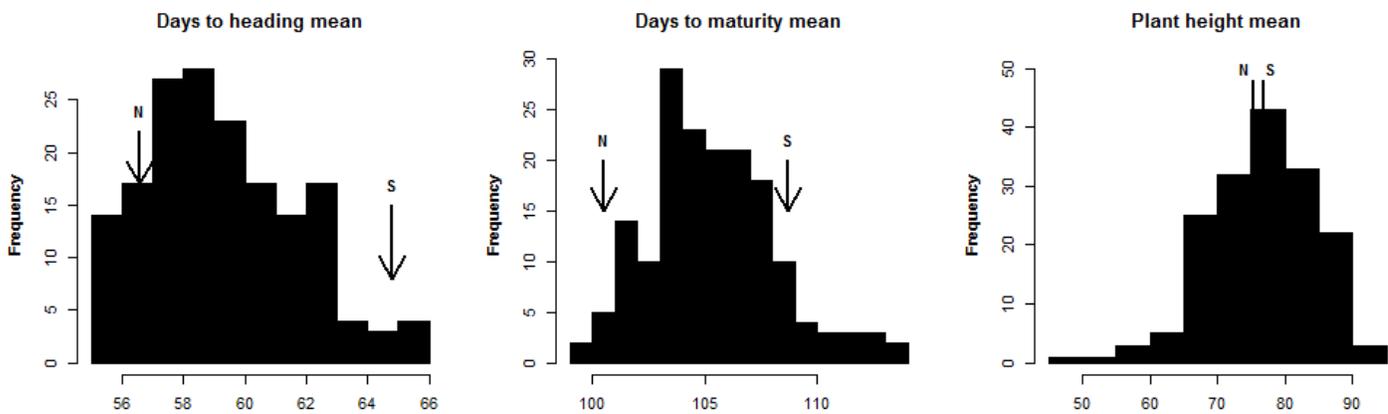


Fig.S1 Frequency distributions of associated traits in the SHA3/CBRD x Naxos RIL population